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Morpho-molecular diversity of Linocarpaceae (Chaetosphaeriales): *Claviformispora* gen. nov. from decaying branches of *Phyllostachys heteroclada*

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Abstract

In this paper, *Claviformispora* **gen. nov.** in Linocarpaceae is introduced from *Phyllostachys heteroclada* in Sichuan Province, China. The new genus is characterised by its distinct morphological characters, such as ostiole with periphyses, asci with a thick doughnut-shaped, J- apical ring and clavate ascospore without septum-like band and appendage. Maximum Likelihood and Bayesian Inference phylogenetic analyses, based on DNA sequence data from ITS, LSU, SSU and TEF-1α regions, provide further evidence that the fungus is a distinct genus within this family. The new genus is compared with similar genera, such as *Linocarpon* and *Neolinocarpon*. Descriptions, illustrations and notes are provided for the new taxon.

Keywords

bambusicolous fungi, one new genus and species, phylogeny, taxonomy

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Introduction

The order Chaetosphaeriales Huhndorf, A.N. Mill. & F.A. Fernández (Sordariomycetes) was introduced in Sordariomycetidae O.E. Erikss. & Winka, based on LSU sequence data (Huhndorf et al. 2004) and currently comprises four families viz. Chaetosphaeriaceae Réblová, M.E. Barr & Samuels, Helminthosphaeriaceae Samuels, Cand. & Magni, Leptosporellaceae S. Konta & K.D. Hyde and Linocarpaceae S. Konta & K.D. Hyde (Hongsanan et al. 2017; Konta et al. 2017; Wijayawardene et al. 2020). Recently, 43 genera were accepted within Chaetosphaeriaceae and seven genera within Helminthosphaeriaceae (Hyde et al. 2020; Wijayawardene et al. 2020). Based on morphology and combined analyses of ITS and LSU sequence data, Konta et al. (2017) accommodated *Linocarpon* Syd. & P. Syd. and *Neolinocarpon* K.D. Hyde in Linocarpaceae and *Leptosporella* Penz. & Sacc. in Leptosporellaceae. Leptosporellaceae and Linocarpaceae are morphologically similar in their dome-shaped ascomata and filiform ascospores (Hyde and Alias 1999; Cai et al. 2004; Konta et al. 2017). The former, however, can be delineated based on ascospores that are narrow, long filiform, with gradually tapering ends and indistinct mucilage (if present), whereas in Linocarpaceae, ascospores have a distinct appendage at the apex and are generally wider and differ in appearance at the ends (Konta et al. 2017).

The genus *Leptosporella* was introduced with *L. gregaria* Penz. & Sacc., 1897 as the type species by Penzig and Saccardo (1897). Lumbsch and Huhndorf (2010) referred it in Sordariomycetidae genera *incertae sedis*. Subsequently, the genus was referred to the Chaetosphaeriales, based on phylogenetic analysis of LSU sequence data (Huhndorf and Miller 2011; Dai et al. 2016; Wijayawardene et al. 2020). At present, 15 epithets of *Leptosporella* are recorded in Index Fungorum (http://www.speciesfungorum.org/Names/Names.asp). Sydow and Sydow (1917) introduced *Linocarpon* with *L. pandani* Syd. & P. Syd., 1917 as the type species. Hyde (1992a) introduced *Neolinocarpon* to accommodate a linocarpon-like species, *N. globosicarpum* K.D. Hyde, 1992. Currently in Index Fungorum (2020), 44 and 13 epithets are accommodated in *Linocarpon* and *Neolinocarpon*, respectively. Hyde (1997) and Jeewon et al. (2003) reported that *Linocarpon* and *Neolinocarpon* can be accommodated in Hyponectriaceae (Xylariales), while Bahl (2006) suggested a closer relationship with Chaetosphaeriales and Xylariales, based on their molecular data. However, with more taxon sampling and fresh collections, Konta et al. (2017) confirmed that *Linocarpon* and *Neolinocarpon* should be accommodated in a distinct family (Linocarpaceae) in Chaetosphaeriales.

The present research is a part of our investigations on the taxonomic and phylogenetic circumscriptions of pathogenic and saprobic micro-fungi associated with bamboo in Sichuan Province, China. In this paper, we introduce a new genus *Claviformispora* in Linocarpaceae, typified by *C. phyllostachydis* from *Phyllostachys heteroclada* Oliv., 1894 (Poaceae). The morphological differences and analyses of a combined ITS, LSU, SSU and TEF-1α sequence dataset support the validity of the new genus and its placement in Linocarpaceae. The new genus is compared with other genera in the family. The comprehensive descriptions and micrographs of new taxa are provided.

Materials and methods

Specimen collection and morphological study

Bamboo materials were collected from Ya'an City, China. Single ascospore isolations were carried out following the method described by Chomnunti et al. (2014) and the germinating spores were transferred to PDA, incubated at 25 °C in the dark and cultural characteristics were determined. Ascomata were observed and photographed using a dissecting microscope NVT-GG (Shanghai Advanced Photoelectric Technology Co. Ltd, China) matched to a VS-800C micro-digital camera (Shenzhen Weishen Times Technology Co. Ltd., China). The anatomical details were visualised using Nikon ECLIPSE *Ni* compound microscope fitted to a Canon 600D digital camera and an OPTEC BK-DM320 microscope matched to a VS-800C micro-digital camera (Shenzhen Weishen Times Technology Co. Ltd., China). Iodine reaction of the ascus wall was tested in Melzer's reagent (MLZ). Lactate cotton blue reagent was used to observe the number of septa. The gelatinous appendage was observed in Black Indian ink. Type specimens were deposited at the Herbarium of Sichuan Agricultural University, Chengdu, China (SICAU) and Mae Fah Luang University Herbarium (MFLU). The ex-type living cultures are deposited at the Culture Collection in Sichuan Agricultural University (SICAUCC) and the Culture Collection at Mae Fah Luang University (MFLUCC). Index Fungorum numbers (http://www.indexfungorum.org/Names/Names.asp) are registered and provided.

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from mycelium that were grown on PDA at 25 °C for two weeks using a Plant Genomic DNA extraction kit (Tiangen, China) following the manufacturer's instructions. The primers pairs LR0R and LR5 (Vilgalys and Hester 1990), NS1 and NS4, ITS5 and ITS4 (White et al. 1990), EF1-983F and EF1-2218R (Rehner 2001) were used for the amplification of the partial large subunit nuclear rDNA (LSU), the partial small subunit nuclear rDNA (SSU), internal transcribed spacers (ITS) and translation elongation factor 1-alpha (TEF-1α), respectively.

Polymerase chain reaction (PCR) was performed in 25 µl final volumes containing 22 µl of Master Mix (Beijing TsingKe Biotech Co. Ltd.), 1 µl of DNA template, 1 µl of each forward and reverse primers (10 µM). The PCR thermal cycle programmes for LSU, SSU, ITS and TEF1-α gene were amplified as: initial denaturation 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 minute and final extension at 72 °C for 10 minutes. PCR products were sequenced with the above-mentioned primers at TsingKe Biological Technology Co. Ltd, Chengdu, China. The newly-generated sequences from the LSU, SSU, TEF-1α and ITS regions were deposited in GenBank (Table 1).

Table 1. Molecular data used in this study and GenBank accession numbers.

Species name	Strain	GenBank accession number			
		LSU	ITS	SSU	TEF
Chloridium aquaticum	MFLUCC 11-0212	MH476567	MH476570	MH476573	–
Chloridium aseptatum	MFLUCC 11-0216	MH476568	NR_158365	MH476574	–
Claviformispora phyllostachydis	**SICAUCC 16-0004**	**MT232720**	**MT232736**	**MT232735**	**MT240855**
Cryptophiale hamulata	MFLUCC 18-0098	MG386756	–	MG386757	–
Cryptophiale udagawae	MFLUCC 18-0422	MH758211	MH758198	MH758205	–
	MFLUCC 18-0428	MH758210	MH758197	MH758204	–
Dictyochaeta siamensis	MFLUCC 15-0614	KX609952	KX609955	–	–
Dictyochaeta assamica	CBS 242.66	MH870426	MH858788	–	–
Dictyochaeta pandanicola	MFLUCC 17-0563	MH376710	MH388338	MH388307	MH388373
Dictyochaeta terminalis	GZCC 18-0085	MN104624	MN104613	MN104633	–
Echinosphaeria canescens	SMH4666	KF765605	–	–	–
	SMH4791	AY436403	–	–	–
Endophragmiella dimorphospora	FMR_12150	KY853502	KY853442	HF937351	–
Gelasinospora tetrasperma	CBS 178.33	DQ470980	NR_077163	DQ471032	DQ471103
Helminthosphaeria clavariarum	SMH4609	AY346283	–	–	–
Hilberina caudata	SMH1542	KF765615	–	–	–
Infundibulomyces cupulata	BCC11929	EF113979	EF113976	EF113982	–
Infundibulomyces oblongisporus	BCC13400	EF113980	EF113977	EF113983	–
Kionochaeta castaneae	GZCC 18-0025	MN104621	MN104610	MN104630	–
Kionochaeta microspora	GZCC 18-0036	MN104618	MN104607	MN104627	–
Leptosporella arengae	MFLUCC 15-0330	MG272246	MG272255	MG366594	MG272259
Leptosporella bambusae	MFLUCC 12-0846	KU863122	KU940134	–	–
Leptosporella cocois	MFLUCC 15-0816	–	MG272256	–	–
Leptosporella gregaria	SMH4290	AY346290	–	–	–
	SMH4673	HM171287	–	–	–
Leptosporella elaeidis	MFLU 19-0669	MK659772	MK659767	MK659774	MN883560
Linocarpon arengae	MFLUCC 15-0331	MG272247	–	MG366596	–
Linocarpon cocois	MFLUCC 15-0812	MG272248	MG272257	MG272253	–
Menispora tortuosa	DAOM 231154	AY544682	KT225527	AY544723	–
	CBS 214.56	AF178558	AF178558	–	–
Menisporopsis anisospora	CBS 109475	MH874421	MH862827	–	–
Menisporopsis breviseta	GZCC 18-0071	MN104623	MN104612	MN104632	–
Menisporopsis dushanensis	GZCC 18-0084	MN104626	MN104615	MN104635	–
Menisporopsis pandanicola	KUMCC 17-0271	MH376726	MH388353	MH388320	MH388388
Menisporopsis theobromae	MFLUCC 15-0055	KX609954	KX609957	–	–
Neolinocarpon arengae	MFLUCC 15-0323	MG272249	MG272258	MG366597	–
Neolinocarpon rachidis	MFLUCC 15-0332	MG272250	–	MG366598	–
	MFLUCC 15-0814a	MK106353	MK106342	MK106367	–
	MFLUCC 15-0814b	MK106354	–	MK106368	
Neolinocarpon phayaoense	MFLUCC 17-0073a	MG581933	–	MG581936	MG739512
	MFLUCC 17-0073b	MG581934	–	MG581937	MG739513
	MFLUCC 17-0074	MG581935	–	MG581938	MG739514
Phialosporostilbe scutiformis	MFLUCC 17-0227	MH758207	MH758194	MH758201	–
	MFLUCC 18-1288	MH758212	MH758199	–	–
Ruzenia spermoides	SMH4606	AY436422	–	–	–
	SMH4655	KF765619	–	–	–
Synaptospora plumbea	ANM963	KF765620	–	–	–
	SMH3962	KF765621	–	–	–
Sordaria fimicola	CBS 508.50	MH868251	MH856730	–	–
Zanclospora iberica	FMR 11584	KY853544	KY853480	HF937360	–
	FMR 12186	KY853545	KY853481	HF937361	–

Notes. New species in this study is in bold. "–" means that the sequence is missing or unavailable.

Abbreviations. ANM: Collection of A.N. Miller; **BCC**: BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok, Thailand; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; **DAOM**: Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; **FMR**: Facultad de Medicina, Universitat Rovira i Virgili, Reus, Tarragona, Spain; **GZCC**: Guizhou Culture Collection, Guiyang, China; **KUMCC**: Kunming Institute of Botany Culture Collection, Chinese Academy of Sciences, Kunming, China; **MFLU**: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **SICAUCC**: Sichuan Agricultural University Culture Collection, Sichuan, China; **SMH**: Collection of S.M. Huhndorf.

Phylogenetic analyses

Taxa to be used for phylogenetic analyses were selected, based on results generated from nucleotide BLAST searches online in GenBank and recent publications (Lu et al. 2016; Konta et al. 2017; Senwanna et al. 2018; Wei et al. 2018; Lin et al. 2019). *Gelasinospora tetrasperma* (CBS 178.33) and *Sordaria fimicola* (CBS 508.50) were selected as the outgroup taxa. The sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) and the accession numbers are listed in Table 1. A combined ITS, LSU, SSU and TEF-1α sequence dataset was used to construct the phylogenetic tree. DNA alignments were performed by using MAFFT v.7.429 online service (Katoh et al. 2019) and ambiguous regions were excluded with BioEdit version 7.0.5.3 (Hall 1999). Multigene sequences were concatenated by using Mesquite software (Maddison and Maddison 2019). Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed. The best nucleotide substitution model was determined by MrModeltest v. 2.2 (Nylander 2004).

Maximum Likelihood analysis and Bayesian Inference analysis were generated by using the CIPRES Science Gateway web server (Miller 2010). RAxML-HPC2 on XSEDE (8.2.10) (Stamatakis 2014) with GTR+GAMMA substitution model with 1000 bootstrap iterations was chosen for Maximum Likelihood analysis. For BI analyses, the best-fit model GTR+I+G for ITS, LSU and SSU was selected in MrModeltest 2.2 and GTR+G for TEF. The analyses were computed with six simultaneous Markov Chain Monte Carlo (MCMC) Chains with 8,000,000 generations and a sampling frequency of 100 generations. The burn-in fraction was set to 0.25 and the run automatically ended when the average standard deviation of split frequencies reached below 0.01.

Phylogenetic trees were visualised with FigTree v.1.4.3 (Rambaut and Drummond 2016) and edited using Adobe Illustrator CS6 (Adobe Systems Inc., United States). Maximum Likelihood bootstrap values (MLBP) equal to or greater than 70% and Bayesian Posterior Probabilities (BYPP) equal to or greater than 0.95 were accepted. The finalised alignment and tree were deposited in TreeBASE (http://www.treebase.org), submission ID: 25996. The new taxa introduced follow the recommendations of Jeewon and Hyde (2016).

Results

Phylogenetic analyses

Phylogenetic analyses of a combined dataset (ITS, LSU, SSU, TEF-1α) comprises 51 taxa within the order Chaetosphaeriales (Table 1), including 24 taxa in family Chaetosphaeriaceae, nine taxa in Helminthosphaeriaceae, ten taxa in Linocarpaceae, six taxa in Leptosporellaceae and two outgroup taxa in Sordariales. The dataset consisted of 5,849 characters including gaps (LSU = 1,571, ITS = 736, SSU = 2,522, TEF = 1,020). The best scoring tree of RAxML analysis is shown in Fig. 1, with the support values of ML and BI analyses.

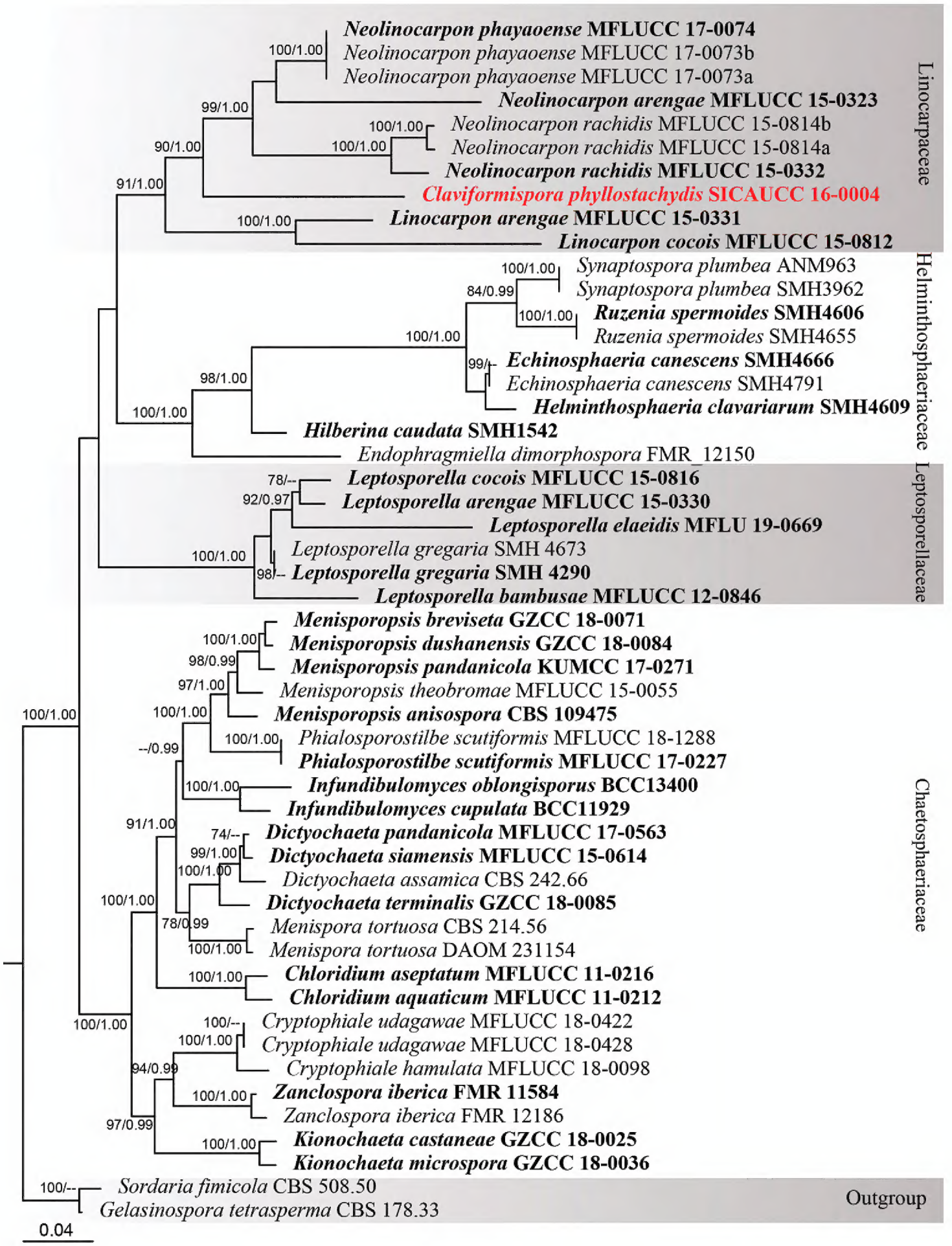

Figure 1. Phylogram of RAxML analysis based on a combined ITS, LSU, SSU and TEF-1α sequence dataset within order Chaetosphaeriales. Bootstrap support values for maximum likelihood (ML, left) greater than 70% and Bayesian posterior probabilities (PP, right) equal to or greater than 0.95 are indicated at the nodes. The tree is rooted to *Gelasinospora tetrasperma* (CBS 178.33) and *Sordaria fimicola* (CBS 508.50). All sequences from ex-type strains are in bold. The newly-generated sequence is in red.

The best scoring RAxML tree with the final optimisation had a likelihood value of -26,415.700648. The matrix had 1,751 distinct alignment patterns and 64.64% in this alignment is the gaps and completely undetermined characters. Estimated base frequencies were as follows: A = 0.236065, C = 0.261532, G = 0.295313, T = 0.207091, with substitution rates AC = 1.062535, AG = 1.855434, AT = 0.940219, CG = 1.052604, CT = 4.590285, GT = 1.000000. The gamma distribution shape parameter $\alpha = 0.311923$ and the Tree-Length = 2.281738. The Bayesian analysis resulted in 20,502 trees after 8,000,000 generations. The first 25% of trees (1,624 trees), which represent the burn-in phase of the analyses were discarded, while the remaining 4,878 trees were used for calculating posterior probabilities. Bayesian posterior probabilities were evaluated by MCMC with a final average standard deviation of split frequencies = 0.009877.

Phylogenetic trees generated from Maximum Likelihood (ML) and Bayesian Inference analyses were similar in overall topologies. Phylogeny from the combined sequence data analysis indicates that all families were monophyletic with strong bootstrap support values (Fig. 1). Phylogenetic results show that our novel species *Claviformispora phyllostachydis* (SICAUCC 16-0004) belongs to family Linocarpaceae with 91% ML and 1.00 BYPP support and close to genera *Neolinocarpon* and *Linocarpon* (Fig. 1). The new genus *Claviformispora* constituted a distinct lineage in the family Linocarpaceae (Fig. 1).

Taxonomy

Linocarpaceae S. Konta & K.D. Hyde, Mycosphere 8(10): 1962 (2017) emend.

Type genus. *Linocarpon* Syd. & P. Syd.

Description. *Saprobic* and *endophytic* fungi on monocotyledons and rarely dicotyledons. ***Sexual morph*:** *Ascomata* solitary or aggregated, superficial or immersed comprising black, dome-shaped or subglobose, slightly raised blistering areas with a central ostiole or immersed with a black shiny papilla. *Peridium* composed of dark brown to black cells of *textura angularis*. *Hamathecium* comprising septate paraphyses that are longer than asci, wider at the base, tapering towards the apex. *Asci* 8-spored, unitunicate, cylindrical, with a J-, apical ring, developing from the base and periphery of the ascomata. *Ascospores* parallel or spiral in asci, hyaline or pale yellowish in mass, filiform or claviform, straight or curved, unicellular with or without refringent bands, with or without polar appendages. ***Asexual morph*:** *Phialophora*-like spp. were found in *Linocarpon appendiculatum* and *L. elaeidis* cultures (Hyde 1992b), but no records are available for other species.

Notes. Linocarpaceae was introduced as a new family to accommodate *Linocarpon* and *Neolinocarpon* species, based on morphology and phylogeny (Konta et al. 2017). Appressoria were first recorded from *Neolinocarpon rachidis* (Hyde et al. 2019). The new genus *Claviformispora*, which is well-supported within Linocarpaceae suggests

that there is a need to amend the morphological circumscriptions of the family given that the ascomata (subglobose) and ascospore (claviform) characters are so different from the other two genera.

Claviformispora **X. L. Xu & C. L. Yang, gen. nov.**
Index Fungorum identifier: IF557395

Type species. *Claviformispora phyllostachydis* X. L. Xu & C. L. Yang

Etymology. Name reflects the claviform ascospores.

Description. *Saprobic* on dead branches. ***Sexual morph*:** *Stromata* solitary or gregarious, black, erumpent. *Ascomata* solitary or aggregated, immersed, subglobose, slightly raised blistering areas with a central ostiole with periphyses. *Peridium* outer cells merging with the host tissues, composed of pale to dark brown cells of *textura angularis*. *Hamathecium* comprising hyaline, septate paraphyses, longer than asci, wider at the base, tapering towards the apex. *Asci* 8-spored, cylindrical to cylindric-clavate, unitunicate, short pedicellate, apically rounded, with a doughnut-shaped, refractive, J- apical ring. *Ascospores* overlapping uniseriate or 2-seriate, clavated with a thin pedicellate, 1-celled, hyaline, without appendage and refringent bands, smooth-walled. ***Asexual morph*:** Undetermined.

Notes. *Claviformispora* resembles *Neolinocarpon* in having immersed ascomata and ostiole with periphyses, but differs in forming aggregated ascomata, cylindric-clavate, short pedicellate asci, clavate ascospores with thin pedicel and without septa-like bands and appendages, whereas the ascospores of *Neolinocarpon* and *Linocarpon* (Linocarpaceae) species are usually filiform with refringent bands and appendages (Hyde 1992b, 1997; Konta et al. 2017). The nature of the ascospore appendages appears to be phylogenetically significant for intergeneric delineation as has been seen in other studies (Poonyth et al. 2000; Jeewon et al; 2003, Thongkantha et al. 2003; Cai et al. 2004; Konta et al. 2017), but this warrants further investigations with more sampling and fresh collections of *Neolinocarpon* and *Linocarpon*. Differences in morphology between these genera in Linocarpaceae are summarised in Table 2.

Claviformispora phyllostachydis **X. L. Xu & C. L. Yang, sp. nov.**
Index Fungorum identifier: IF557396
Fig. 2

Type. China, Sichuan Province, Ya'an City, Yucheng Distinct, Kongping Township, alt. 1133 m, 29°50.14'N, 103°03'E, on dead branches of *Phyllostachys heteroclada* Oliv. (Poaceae), 11 December 2016, C. L. Yang and X. L. Xu, YCL201612002 (SICAU 16-0007, ***holotype***; MFLU 18-1217, ***isotype***), ex-type living culture, SICAUCC 16-0004 = MFLUCC 18-1230.

Etymology. The specific epithet refers to the host genus *Phyllostachys*.

Description. *Saprobic* on dead branches of *Phyllostachys heteroclada* Oliv. ***Sexual morph*:** *Stromata* solitary comprising elliptical areas or aggregated in large black

Table 2. Morphological comparison of *Linocarpon*, *Neolinocarpon* and *Claviformispora*.

Morphology	***Linocarpon*** (Type: ***L. pandani***)	***Neolinocarpon*** (Type: ***N. globosicarpum***)	***Claviformispora*** (Type: ***C. phyllostachydis***)
Stromata	Absent	Absent	Solitary or aggregated, comprising elliptical areas and large black areas, with slit-like openings
Ascomata	Solitary, superficial, subglobose, comprising black, dome-shaped, raised blistering areas, central ostiole	Solitary, deeply immersed, oval to globose, with central raised, dark, shiny papilla, central ostiole with periphyses	Solitary or aggregated, deeply immersed, subglobose, slightly raised blistering areas, central ostiole with periphyses
Peridium	Textura angularis	Textura angularis	Textura angularis
Hamathecium	Hyaline, septate paraphyses, longer than asci	Hyaline, septate paraphyses, longer than asci	Hyaline, septate paraphyses, longer than asci
Asci	Cylindrical, unitunicate, a small non-amyloid apical ring	Long cylindrical, pedicellate, unitunicate, an oblong to wedge-shaped, refractive, apical ring and some with a refractive circular body below	Cylindrical to cylindric-clavate, unitunicate, pedicellate, doughnut-shaped, refractive, J- apical ring
Ascospores	Filiform, aseptate, hyaline or pale-yellowish in mass, parallel or spiral, with appendage and refringent septum-like bands or absent	Filiform, aseptate, hyaline or pale-yellowish in mass, parallel or spiral, with apical appendages and refringent bands or absent	Clavate, thin pedicellate, aseptate, hyaline, parallel, no appendage and refringent band
Asexual morph	Only found in *L. appendiculatum* and *L. elaeidis*, conidiophore arising from the aerial mycelium, conidiogenous cells phialidic, smooth, translucent brown, conidia clavate to fusiform, straight or slightly curved or slightly sinuous, unicellular, smooth, colourless	Undetermined	Undetermined
Others	Colonies on MEA and PDA growing slowly	Colonies on MEA growing slowly. Ascospores on MEA produced appressoria-like structures at each tip of germ tube, only found in *N. rachidis*	Colonies on PDA grow faster
References	Hyde (1992b), Konta et al. (2017), Thongkantha et al. (2003)	Hyde et al. (2019), Senwanna et al. (2018), Hyde et al. (1998)	This study

areas, slightly raised with slit-like openings presenting on host surface. *Ascomata* 120–240 μm high × 220–490 μm diameter ($\bar{x}$ = 189 × 345 μm, n = 20), perithecial, immersed, central, papillate ostiole with periphyses, oval-globose in section, the cells between the perithecia are composed with tissue of stromata and host. *Peridium* 20–40 μm wide ($\bar{x}$ = 33 μm, n = 10), outer cells merging with the host tissues, composed of pale to dark brown cells of *textura angularis*. *Hamathecium* comprising hyaline, hypha-like, septate paraphyses, occasionally branched, longer than asci, wider at the base, 2–4 μm diameter ($\bar{x}$ = 2.7 μm, n = 20) tapering towards the apex, 0.78–1.20 μm diameter ($\bar{x}$ = 0.98 μm, n = 20). *Asci* 90–160 × 9–15 μm ($\bar{x}$ = 118 × 13 μm, n = 20), 8-spored, cylindrical to cylindric-clavate, unitunicate, short pedicellate, apically rounded, with a massive, doughnut-shaped, refractive, J- reaction, apical ring. *Ascospores* 35–50 × 5.7–8.6 μm ($\bar{x}$ = 45.7 × 7.0 μm, n = 40), overlapping uniseriate or 2-seriate, claviform typically with a thin pedicel, aseptate, hyaline, straight or slight curved, without appendage and septum-like bands, guttulate when maturity. ***Asexual morph*:** Undetermined.

Culture characters. Ascospores germinated on PDA within 12 hours at both ends. Colonies on PDA reaching 5 cm diameter after 7 days at 25 °C, white to grey with strong radiations outwards on forward side. Colonies became dark brown and black on the reverse after a long time of cultivation. The hyphae are septate, branched, smooth.

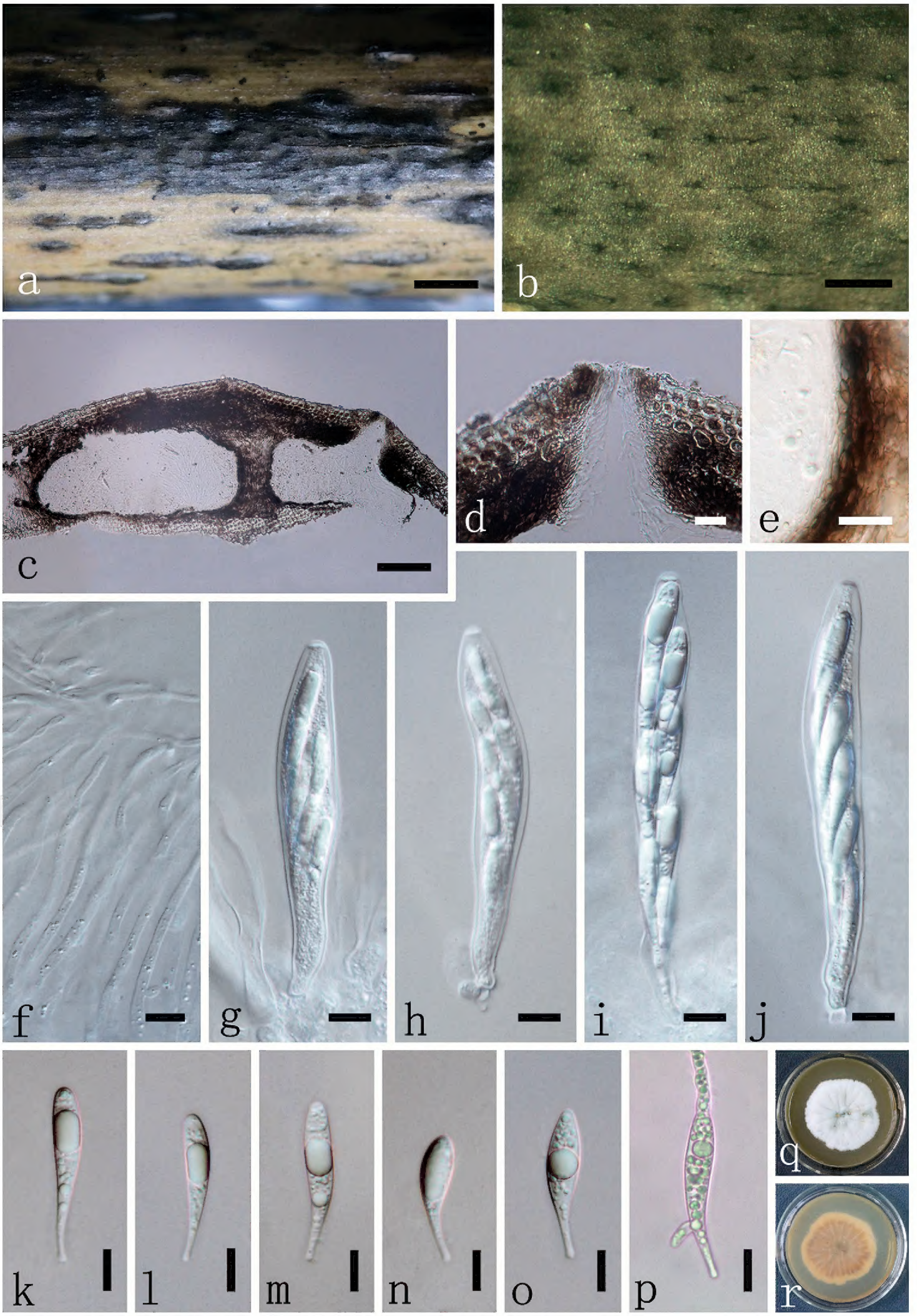

Figure 2. *Claviformispora phyllostachydis* (SICAU 16-0007, holotype) **a, b** *Stromata* on host substrate **c** section through ascoma with ascomata **d** ostiole with periphyses **e** peridium **f** paraphyses **g–j** asci **k–o** ascospores **p** germinated ascospore **q, r** colony on PDA after 7 days. Scale bars: 2 mm (**a**), 500 μm (**b**), 100 μm (**c**), 20 μm (**d, e**), 10 μm (**f–p**).

Discussion

This study establishes a new genus and also provides further insights into the phylogeny of members associated with Linocarpaceae. Morphologically-based examinations of *Claviformispora* (as discussed above) clearly show that the morphological circumscriptions (familial concept) of species should be broadened and possibly indicate that this family is much more diverse than expected. Our collection can be clearly distinguished from other groups of similar fungi in Linocarpaceae with its interesting ascospore morphology. In addition, we also noted some peculiarities in the DNA sequences we analysed. A comparison of ITS sequences, based on BLAST reveals 34%, 26% and 30% base pair differences with *L. cocois* (MFLUCC 15-0812), *N. arengae* (MFLUCC 15-0323) and *N. rachidis* (MFLUCC 15-0814a), respectively. There are more than 9% and 5% sequence differences with the three taxa when the LSU and SSU rDNA sequences were compared respectively. Following the guidelines recommended by Jeewon and Hyde (2016), there are therefore sufficient grounds to establish a new species at the genus rank.

Species of Linocarpaceae have been found on Arecaceae, Poaceae, Euphorbiaceae, Zingiberaceae, Pandanaceae, Fagaceae, Fabaceae and Smilacaceae, including *Arenga*, *Attalea*, *Calamus*, *Trachycarpus*, *Acrocomia*, *Archontophoenix*, *Cocos*, *Daemonorops*, *Licuala*, *Livistona*, *Plectocomia*, *Phoenix*, *Raphia*, *Sabal*, *Mauritia*, *Nypa*, *Elaeis*, *Pinanga*, *Eugeissona*, *Pennisetum*, *Gramineae*, *Stipa*, unidentified bamboo, *Hevea*, *Manihot*, *Alpinia*, *Pandanus*, *Quenrcus*, *Cajanus* and *Smilax* (Hyde 1988, 1992a, b; Dulymamode et al. 1998; Hyde et al. 1998; Hyde and Alias 1999; Thongkantha et al. 2003; Cai et al. 2004; Bhilabutra et al. 2006; Vitoria et al. 2013; Konta et al. 2017; Senwanna et al. 2018). More than 50% of the species were recorded from hosts of the Arecaceae. Species in Linocarpaceae are mostly saprobic, except *Linocarpon palmetto* which was discovered as a pathogen of *Sabal palmetto* in Florida (Barr 1978). Four species in Linocarpaceae from Poaceae have been reported so far, including *Neolinocarpon penniseti* on *Pennisetum purpureum* (Bhilabutra et al. 2006), *Linocarpon williamsii* on *Gramineae* sp. (Hansford 1954), *L. stipae* on *Stipa* sp. (Hansford 1954) and *L. bambusicola* on unidentified bamboo submerged in a river (Cai et al. 2004).

Phyllostachys heteroclada, mainly a food source and use as a material in the weaving industry, is distributed along the Yellow River Valley and the southern Provinces in China. It is common in the mountainous areas of Sichuan Province with distribution up to 1,500 m above sea level (Yi 1997; Yi et al. 2008). There is a large area of pure forest in Yibin, Leshan and Ya'an Cities and sporadic distribution in other areas. According to preliminary statistics, bambusicolous fungi from seven orders (excluding fungi referred to as Sordariomycetes *incertae sedis*) have been recorded on *P. heteroclada*, including Hypocreales, Ostropales, Pleosporales, Phyllachorales, Pucciniales, Ustilaginales and Xylariales, of which Pleosporales is the largest one. Most bambusicolous fungi in China were recorded with inadequate morphological descriptions or molecular data. The early known fungi on *P. heteroclada* are documented as *Aciculosporium take*, *Ellisembia pseudoseptata*, *Fusarium oxysporum*, *F. semitectum*, *Phyllachora gracilis*, *Ph. orbicular*, *Shiraia bambusicola*, *Stereostratum corticioides* and *Ustilago shiraiana* (Zhou et al. 2001; Xu et al. 2006). In recent years, some new records and taxa, viz. *Bambusicola subthailandica*,

B. sichuanensis, *Neostagonosporella sichuanensis*, *Parakarstenia phyllostachydis*, *Phyllachora heterocladae*, *Podonectria sichuanensis*, *Arthrinium yunnanum* and *A. phyllostachium* have been reported (Yang et al. 2019a, b, c, d, e, f). Here, we introduce a new genus in order Chaetosphaeriales, which is a contribution to fungal diversity on *P. heteroclada*.

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Supplementary material 1

Figure S1

Authors: Xiu-Lan Xu, Chun-Lin Yang, Rajesh Jeewon, Dhanushka N. Wanasinghe, Ying-Gao Liu, Qian-Gang Xiao

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Supplementary material 2

Figure S2

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